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Novel HIV IL-4R antagonist vaccine strategy can induce both high avidity CD8 T and B cell immunity with greater protective efficacy

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ABSTRACT

We have established that the efficacy of a heterologous poxvirus vectored HIV vaccine, fowlpox virus (FPV)-HIV gag/pol prime followed by attenuated vaccinia virus (VV)-HIV gag/pol booster immunisation, is strongly influenced by the cytokine milieu at the priming vaccination site, with endogenous IL-13 detrimental to the quality of the HIV specific CD8+ T cell response induced. We have now developed a novel HIV vaccine that co-expresses a C-terminal deletion mutant of the mouse IL-4, deleted for the essential tyrosine (Y119) required for signalling. In our vaccine system, the mutant IL-4C118 can bind to IL-4 type I and II receptors with high affinity, and transiently prevent the signalling of both IL-4 and IL-13 at the vaccination site. When this IL-4C118 adjuvanted vaccine was used in an intranasal rFPV/intramuscular rVV prime-boost immunisation strategy, greatly enhanced mucosal/systemic HIV specific CD8+ T cells with higher functional avidity, expressing IFN-γ, TNF-α and IL-2 and greater protective efficacy were detected. Surprisingly, the IL-4C118 adjuvanted vaccines also induced robust long-lived HIV gag-specific serum antibody responses, specifically IgG1 and IgG2a. The p55-gag IgG2a responses induced were of a higher magnitude relative to the IL-13Rα2 adjuvant vaccine. More interestingly, our recently tested IL-13Rα2 adjuvanted vaccine which only inhibited IL-13 activity, even though induced excellent high avidity HIV-specific CD8+ T cells, had a detrimental impact on the induction of gag-specific IgG2a antibody immunity. Our observations suggest that (i) IL-4 cell-signalling in the absence of IL-13 retarded gag-specific antibody isotype class switching, or (ii) IL-13Rα2 signalling was involved in inducing good gag-specific B cell immunity. Thus, we believe our novel IL-4R antagonist adjuvant strategy offers great promise not only for HIV-1 vaccines, but also against a range of chronic infections where sustained high quality mucosal and systemic T and B cell immunity are required for protection.

1. Introduction

Many licensed human vaccines are currently available for a wide range of bacterial and viral diseases; unfortunately it is evident that conventional approaches to developing vaccines against chronic and challenging diseases such as HIV/AIDS, malaria or tuberculosis do not induce the correct "type" or level of immunity sufficient to prevent infection or control of the disease. This highlights the importance of developing innovative vaccine approaches that can induce sufficiently high level of protective immunity [1]. Surprisingly, thirty years have passed since the discovery of HIV and the exact correlates of the immune responses that potentially protect against HIV infection or attenuate the development of AIDS are still poorly understood. The development of an effective vaccine against HIV/AIDS will require an in-depth understanding of the antiviral immunity to HIV-1 and identifying and engineering the desirable types of immunity required for protective efficacy [2]. For example, understanding the mechanisms by which HIV evades the immune system and tailoring the immunity to counteract such immune escape may be of importance. In addition, an in-depth understanding of viral vaccine vectors utilised and how the vector's own intrinsic genetics and products influence the development of the immune response needs to be understood to maximise vaccine efficacy. These features have been largely ignored in previous vaccine trials resulting in unexpected vaccine failures (e.g. Adenovirus-based STEP trial).

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Multiple HIV-1 vaccines have been trialled over recent decades that although yielding good immune outcomes in animal models have disappointingly failed to induce protective immunity in human clinical trials. Both the Adenovirus vectored HVTN505 and the previous STEP vaccine trials were prematurely aborted due to significant numbers of vaccine subjects becoming infected with HIV [3]. The Thailand RV144 trial which used a canarypox virus prime expressing HIV gag, pol and env (ALVAC) followed by a protein booster with recombinant envelope gp120 and adjuvant (AIDSVAC B/E) is the only vaccine to date to show any encouraging results with a modest 31.2% protection [4]. Interestingly, these two vaccines when given individually failed to induce significant immunity in humans [5,6]. Subsequent studies of the RV144 trial data indicated that antibody-dependent cell-mediated cytotoxicity (ADCC) [7] and antibodies directed towards the V1/V2 region of env may contributed to the protective immunity observed [8–10]. Interestingly, no neutralising antibodies or CD8 T cell mediated immunity were detected in this trial, which may explain the partial protection observed [4]. Since the RV144 trial, much of the current HIV vaccine research efforts have been directed towards inducing similar HIV-specific humoral immunity. Nonetheless, any successful future vaccine should also include the ability to induce high quality T cell mediated immunity for effective protective efficacy. Notably, in HIV elite controllers the reduced viral loads have been associated with cell-mediated immunity. Recent studies have shown that the HIV elite controllers have elevated numbers of high avidity polyfunctional cytotoxic HIV Gag-specific CD8+ T-cells in the mucosae compare to the HIV progressors [11–13].

HIV transmits mostly via the genital tract or rectal mucosa and the first CD4 T cell depletion occurs in the gut mucosae [14]. It is now established that HIV is a disease of the mucosae, thus a mucosal vaccine approach may prove more useful in preventing and controlling HIV infection [15,16]. Unfortunately, due to the complexities associated with delivery, safety and evaluation of vaccines efficacy in the mucosae, no mucosal HIV vaccine strategy has yet entered clinical development. Belyakov and co-workers have demonstrated that the intra-rectal immunisation induces local mucosal compartmentalisation of CTL of high "functional avidity" and protection of gastrointestinal CD4+ T cells from SHIV viral depletion in rhesus macaques compared to systemic delivery [17,18]. Consistent to their finding we have also found that i.m. rDNA/i.n. rFPV can induce improved protection in macaques [19]. Since then in our laboratory we have studied the immune outcomes induced following mucosal and systemic heterologous prime-boost vaccination of antigenically distinct poxvirus vectors, Avipoxvirus fowlpox virus (FPV)-HIVgag/pol prime followed by an attenuated Orthopoxvirus vaccinia virus (VV)-HIVgag/pol booster vaccination [20]. These studies have shown that according to the route of vaccine delivery the quality or avidity of HIV-specific CD8 T cells can be vastly different and specifically, IL-13 and IL-4 have an inhibitory influence upon the development of high avidity CD8+ T cell responses. Our data has demonstrated that (i) mucosal vaccination can induce high avidity HIV-specific CD8+ T cells with reduced IL-4/IL-13 activity and better protective efficacy [21], (ii) IL-13 in the cell milieu has a direct negative impact upon CD8+ T cell avidity [22] and (iii) direct neutralisation of endogenous IL-13 activity using a high affinity cytokine receptor, IL-13Rα2 adjuvanted HIV vaccines delivered intranasal/intramuscular strategy can induce high avidity systemic and mucosal HIV-gag specific CD8+ T cell responses, with enhanced cytokine/chemokine expression and greater protective efficacy [23]. Surprisingly, transient inhibition of IL-13 activity at the site of immunisation in wild-type mice generated similar CD8+ T cell responses in regards to avidity and anti-viral protection as IL-13$^{-/-}$ gene knockout mice immunised with control vaccines [23].

Cytokines IL-4 and IL-13 share sequence similarity, cell surface receptor subunits, intracellular signalling and relatively similar functional effects on cells. The IL-4 receptor exists in two forms, the IL-4Rα chain which directly binds IL-4 is a common receptor component for both the IL-4R type I (IL-4Rα/γc) and type II (IL-4Rα/IL-13Rα1), the latter complex also functions as the receptor for IL-13 [24]. Cellular distribution of the receptors differs with the type I receptor generally expressed by hematopoietic cells and type II by non-hematopoietic cells due to differing expression of the γc and IL-13Rα1 subunits, while macrophages express both type I and II receptors. Engagement of IL-4/IL-13 to the receptors triggers cell signalling via JAK/STAT6 dependent mechanisms [25]. A second receptor, IL-13Rα2, binds IL-13 with high affinity and is thought to be a decoy receptor sequestering IL-13 [24], although some studies suggest an uncharacterised signalling activity [26]. Previously, Ahlers et al. [27] demonstrated that soluble IL-13Rα2-Fc decoy receptor together with GM-CSF and CD40L as molecular adjuvants can enhance magnitude HIV Env-specific CD8+ CTL peptide vaccine response. However, IL-13Rα2-Fc protein used alone without other co-stimulators failed to enhance CTL magnitude or activity. Consistent with this finding we have also found that, a single administration of soluble IL-13Rα2-Fc protein together with FPV-HIV made no difference in HIV-specific CD8+ T cell numbers or T cell avidity [23]. In contrast, HIV vaccines co-expressing IL-13Rα2 decoy receptor was able to sequester free IL-13 and greatly enhance magnitude, functional avidity and poly-functionality of the HIV Gag-specific CD8+ T cell response [23].

A number of IL-4 derivatives that either mutate or delete the essential tyrosine residue found in the C-terminal region of both human and mouse cytokines have been developed which bind to cellular IL-4Rα with high affinity without stimulating cell signalling and block activation by both endogenous IL-4 and IL-13 [28–31]. To avoid introducing novel viral expressed "IL-4 antigens" due to amino acid substitutions we have constructed recombinant FPV and VV co-expressing a soluble mouse IL-4 protein containing a short C-terminal deletion encompassing the essential Y119, IL-4C118, while retaining high affinity binding to both IL-4R types I and II and blocking IL-4/IL-13 cell signalling (see Suppl. Diagram 1).

In this study we have evaluated the efficacy of this novel IL-4R antagonist HIV vaccine, specifically the ability to not only induce high avidity CD8+ T cells but also B cell immunity. In this study the HIV-specific T cell responses were evaluated against the BALB/c $Gag_{197–205}$ AMQMLKETI immune-dominant CD8 T cell epitope [32]. As we have previously shown that CD8+ T cells specific for the immuno-dominant epitope represent approximately 80% of the total Gag response in an FPV-HIV/VV-HIV immunisation setting [33]. The B cell responses were measured against the total HIV P55 Gag protein.

2. Materials and methods

2.1. RT-PCR isolation of IL-4C118 cassette and DNA cloning

The mouse IL-4C118 cDNA was isolated using the reverse transcriptase polymerase chain reaction (RT-PCR) method and the Qiagen RT-PCR kit to amplify the cDNA from mouse spleen total RNA. The following gene specific primers were used to isolate the IL-4C118 cDNA; forward primer 5′-GGATCCACCATGGGTCTCAACCCCCAGCTA, containing a terminal BamHI restriction site and a consensus Kozak sequence overlapping the IL-4 methionine codon (underlined) and reverse primer 5′-GAATTCTAATCCATTTGCATGATGCTC, containing a terminal EcoRI site at the end and introduces a stop codon (underlined complimentary sequence) to prematurely truncate the protein

Table 1
Prime boost vaccine strategies used in this study.

	Prime	Boost
1	i.n. FPV-HIV	i.m. VV-HIV
2	i.n. FPV-HIV-IL-4C118	i.m. VV-HIV
3	i.n. FPV-HIV	i.m. VV-HIV-IL-4C118
4	i.n. FPV-HIV-IL-4C118	i.m. VV-HIV-IL-4C118
5	i.n. FPV-HIV-IL-13Rα2Δ10[a]	i.m. VV-HIV-13Rα2Δ10[a]

All rFPV and rVV constructs encode HIV-1 gag/pol antigens originating from (FPV086 and VV336 respectively) [37]. i.n. intranasal, i.m. intramuscular.

[a] Note that for the IL-13Rα2 vaccine used here is the soluble form that was used in our previous study [23].

prior to tyrosine residue Y119. The resulting mutant protein contained a C-terminal aspartic acid at position 118 (IL-4C118) of the mature protein following cleavage of the N-terminal signal peptide. The 431 bp cDNA PCR fragment was ligated into pDrive vector (Qiagen) and confirmed by DNA sequencing. The IL-4C118 cDNA was ligated between the BamHI and EcoRI sites of the VACV vector pTK7.5A [34]. The pTK7.5A vector contains the herpes simplex virus thymidine kinase (tk) gene as a selectable marker. The IL-4C118 cDNA was ligated into pBluscriptSK+ (Promega) and then excised as a BamHI–HindIII fragment and ligated into the multiple cloning site of the FPV vector pAF09 [35]. The IL-4 methionine codon was positioned in-frame with the ATG of the poxvirus late promoter contained in pAF09 to maximise translation. The pAF09 vector contains the *Escherichia coli* gpt gene to enable growth selection in the presence of mycophenolic acid and xanthine, and the lacZ gene for colour selection of recombinant viral plaques.

2.2. Isolation of recombinant viruses

Recombinant poxviruses were constructed essentially as described [36] and briefly described here. Recombinant VV336 contains the insertion of the HIV gag/pol(mut) genes into VV tk gene causing the virus to have a TK-negative phenotype [37]. A recombinant VV co-expressing HIV gag/pol and IL-4C118 was constructed by transfection of VV336 infected HuTK-143B (ATCC CRL8303) cells with pTK7.5A-IL-4C118 using Lipofectamine 2000 transfection reagent (Invitrogen). Recombinant viruses expressing the herpes simplex virus TK were isolated using HuTK-143B cells and culture media containing HAT supplement (Sigma). Recombinant FPV were similarly constructed and isolated using parent virus FPV086, which expresses the HIV gag/pol protein [37], grown on primary chicken embryo skin (CES) cells transfected with pAF09-IL4C118. Recombinant FPV were selected and isolated in culture media containing mycophenolic acid, xanthine and 1x HAT supplement to select for co-expression of the *E. coli* gpt gene. Recombinant viral plaques were identified for co-expression of the *E. coli* lacZ gene using an agarose overlay containing 200 μg/ml X-gal [35,38]. Insertion and expression of the mouse IL-4C118 gene was confirmed by PCR for the inserted DNA sequence and immuno-blotting for secreted IL-4 protein (see Suppl. Fig. 1).

2.3. Immunisation of mice and protection against surrogate recombinant influenza-HIV challenge

Pathogen free 6–7 week old female BALB/c (H-2d) mice were obtained from the Animal Breeding Establishment, John Curtin School of Medical Research (JCSMR). All animals were maintained and used in accordance with the Australian National University (ANU) animal experimentation ethics committee approved guidelines. Mice ($n = 4$–8 per group) were prime-boost immunised i.n./i.m. with 1×10^7 plaque forming units (PFU) rFPV followed by 1×10^7 PFU rVV expressing HIV-1 antigens and IL-13Rα2 or IL-4C118 antagonist as described in Table 1 under mild methoxyfluorane anaesthesia two weeks apart. Similarly groups of mice were used as unimmunised controls. Immediately prior to delivery the viruses were diluted in phosphate buffered saline (PBS) and sonicated 20–30 s to obtain an homogeneous viral suspension, intranasal rFPV was given in a final volume of 20–25 μl and i.m. rVV were delivered, 50 μl per quadriceps.

To evaluate CD8 T cell mediated protective immunity, 6 weeks post booster vaccination, immunised and unimmunised mice were challenged intranasally with 75–100 PFU of influenza virus PR8 expressing the KdGag$_{197–205}$ epitope of HIV as described previously [23]. Body weight was monitored for 9–10 days after challenge. The attenuated recombinant influenza virus PR8-K^{d}Gag$_{197–205}$ incorporates the H2-K^d restricted immuno-dominant epitope AMQMLKETI [32] into the influenza virus neuraminidase stalk, constructed as described by Cukalac et al. [39]. Intra-nasal challenge of naïve BALB/c (H2-K^d) mice with PR8-K^{d}Gag$_{197–205}$ induces significant weight loss, followed by weight gain as the mice recover from a mild flu, over a 10 day period. The cells infected with PR8-K^{d}Gag$_{197–205}$ present the MHC-I restricted HIV-Gag epitope, and in HIV Gag immunised mice CD8+ CTL specific for HIVGag$_{197–205}$ will kill the infected cells limiting replication and dissemination of the recombinant influenza virus significantly reducing weight loss. The ability to maintain body weight specifically at peak infection (4–7 days) is considered a measure of CD8+ T cell mediated protective immunity not antibody immunity.

2.4. Preparation of mucosal and systemic lymphocytes

To measure systemic and mucosal T cell responses mice were euthanized at different time intervals (2 and 8 weeks) post-boost immunisation, and 10 days post influenza-KdGag$_{197–205}$ challenge; spleen, genito-rectal nodes (GN) or iliac lymph nodes and Peyer's patch (PP) were removed and cell suspensions prepared in complete (5% FBS) RPMI as described previously [20,40,41].

2.5. Tetramer staining and dissociation assays

Allophycocyanin-conjugated KdGag$_{197–205}$ tetramers were synthesised at the Bio-Molecular Resource Facility at The John Curtin School of Medical Research (BRF/JCSMR), ANU. 2–5 $\times 10^6$ splenocytes or mucosal lymphocytes were stained with anti-CD8-FITCα antibody (Biolegend, USA) and Allophycocyanin-conjugated KdGag$_{197–205}$ tetramer at room temperature and analysed as described previously [20,40,42]. All the appropriate controls were performed and the background tetramer counts in naïve mice were found to be between 0.05 and 0.5% in spleen, 0.02–0.05% in mucosal tissue and GN. Also following KdGag$_{197–205}$ tetramer staining the dissociation assays were performed as described before [21,43]. In these assays, plates were configured to include five time points per sample (0–60 min). 50 μg/ml of anti-H-2Kd competitive binding antibody (BD PharMigen, San Diego, USA) was added to each well to prevent dissociated tetramer from re-binding and plates were incubated at 37 °C, 5% CO_2. At each time point, cells were transferred into ice-cold FACS buffer to stop the reaction, washed and resuspended in 100 μl of FACS buffer containing 0.5% paraformaldehyde. 100,000 events were acquired on a FACs Calibur flow cytometer (Becton-Dickinson, San Diego, USA) and analysed using Cell Quest Pro software. In tetramer dissociation assays, lower dissociation rates or stronger MHC-I/peptide complex binding to the TCR complex of CD8 T cell, is associated with higher avidity [43].

2.6. IFN-γ and IL-2 ELISpot assays

IFN-γ or IL-2 capture ELISpot assays was used to assess IFN-γ or IL-2 HIV-specific T cell responses [40]. Briefly, 2 $\times 10^5$ spleen or GN cells were added to 96-well Millipore PVDF plates (Millipore, MA, Ireland) coated with 5 μg/ml of mouse anti-IFN-γ or IL-2

capture antibodies (BD PharMigen, San Diego, CA), and stimulated for 12 h or 22 h respectively for IL-2 or IFN-γ ELISpot, in the presence of H-2Kd immuno-dominant CD8+ T cell epitope, $Gag_{197-205}$ AMQMLKETI (synthesised at the Bio-Molecular Resource Facility at JCSMR). ConA-stimulated cells (Sigma, USA) were used as positive controls and unstimulated cells as negative controls. For both ELISpot assays, all steps were carried out exactly as described previously [20,40]. The graphed data are expressed as SFU (spot-forming units) per 10^6 T cells and represent mean values ± SD. Unstimulated cell counts were subtracted from each stimulated value before plotting the data. In all assays the background SFU counts were between 4–10 SFU for IFN-γ and 5–18 SFU for IL-2 ELISpot. Also the unimmunised animals did not show any responses following $Gag_{197-205}$-AMQMLKETI stimulation.

2.7. Intracellular cytokine staining (ICS)

IFN-γ and TNF-α producing HIV-specific CD8 T cells, were analysed as described in Ranasinghe et al. [20,40]. Briefly, 2×10^6 lymphocytes were stimulated with AMQMLKETI peptide at 37 °C for 16 h, and further incubated with Brefeldin A (eBioscience, CA, USA) for 4 h. Cells were surface-stained with CD8-Allophycocyanin (Biolegend, USA) then fixed and permeabilized prior to intracellular staining with IFN-γ-FITC and TNF-α-PE (Biolegend, USA). Total 100,000 gated events per sample were collected using FACS Calibur flow cytometer (Becton Dickinson, San Diego, CA), and results were analysed using Cell Quest Pro software. Prior to plotting the graphs the unstimulated background values were subtracted from the data, The IFN-γ+ cell counts were less than 0.05–0.1% in unimmunised or unstimulated samples similar to our previous studies [23].

2.8. HIV-1 P55 Gag-specific serum enzyme-linked immunosorbent assay (ELISA)

Female BALB/c mice $n = 8$ were i.n./i.m. prime-boost immunised using the strategies 1, 4 and 5 indicated in Table 1. ELISA was used to determine HIV-1 p55 gag-specific IgG1 and IgG2a serum antibody titres similar to as described in Ranasinghe et al. [40]. Falcon microtest III plates were coated with HIV-1 p55-gag (supplied by the NIH AIDS research and reference reagent programme) at 1 μg/ml (50 μl/well) in borate buffer (Pierce) overnight at 4 °C. The plates were washed 5 times with 0.05% Tween20 in PBS (PBST) and nonspecific binding sites were blocked by adding 200 μl of 5% skim milk in PBST incubated at 37 °C for 2 h. The plates were then washed as before, the serum samples were then diluted 2 fold (IgG1 1:800–1:25600, IgG2a 1:200–1:6400) in 5% skim milk/PBST and 50 μl added to each well. The plates were incubated at 37 °C for 4 h washed as before, secondary antibody, biotin-conjugated goat anti-mouse IgG1 or goat anti-mouse IgG2a (Southern Biotechnology Associates, Birmingham, AL) was diluted to 1:500 in 1% bovine serum albumin/PBST (Sigma) (BSA/PBST) was added to respective wells in a 50 μl volume, and incubated overnight at 4 °C. The plates were washed in PBST and 50 μl of streptavidin horseradish peroxidise (Amersham Biosciences) diluted 1:500 in 1% BSA/PBST was added and incubated at 37 °C for 2 h. Next the antibodies were detected using 0.01 mg/ml tetramethyl-benzidine (Sigma) substrate dissolved in dimethyl sulfoxide (Sigma) diluted in citrate/phosphate substrate buffer (Sigma) and incubated for 30 min at room temperature. The optical densities (OD) were measured at 405 nm using a Tecan Infinite m200 Pro Spectrometer.

2.9. Statistics

For T cell-based assays SD or SEM were calculated and p-values determined using a two-tailed, two sample equal variance or unequal variance Student's t-test or one-way ANOVA to compare the groups, followed by post hoc analysis with Sidak multiple comparison test using IBM, SPSS (formerly known as Statistical Package for the Social Sciences) statistical software version 21. Except where stated, experiments were repeated at least three times. To determine endpoint titres, serum from unimmunised mice was titrated across an ELISA plate beginning at the same dilution as the samples. The samples were considered as positive when the OD was at least 3 times the unimmunised mouse serum. Sample that were found not be positive were assigned the titre of half the first dilution. The serum antibody responses were compared using the Mann–Whitney U test was performed using Prism software version 6.02 (Graphpad Inc.) and these antibody experiments were repeated two times.

3. Results

3.1. HIV-1 vaccines that co-express IL-4R antagonist induces high avidity CD8+ T cell immunity

When BALB/c mice were i.n./i.m. prime-boost immunised using the IL-4R antagonist vaccine as described in Table 1 (strategies 2–4), and the CD8+ T cell avidity was evaluated using tetramer dissociation assays, which is a direct measurement of the binding strength of the tetramer MHC-I/peptide to the TCR/complex of the HIV-specific CD8 T cell. This measurement is independent of the numbers of tetramer positive cells in the sample or the ability of a CD8 T cell to express IFN-γ upon peptide stimulation. (Note: high avidity CD8 T cells the dissociation rate is slower compared to low avidity CD8 T cells which shows a higher dissociation rate [21,43]), The data indicated that FPV-HIV-IL-4C118/VV-HIV-IL-4C118 immunisation induced $K^dGag_{197-205}$-specific CTL of higher avidity compared to control vaccination ($p = 0.01$) (Fig. 1). Moreover, the data again demonstrate that inclusion of the antagonist in the prime, and not the booster, was essential for the generation of high avidity T cells (FPV-HIV/VV-HIV vs. FPV-HIV-IL-4C118/VV-HIV) ($p = 0.025$), as inclusion of the IL-4R antagonist in the booster induced $K^dGag_{197-205}$-specific CTL that were of similar avidity to control vaccination (Fig. 1). These results are similar to that of IL-13Rα2 adjuvanted vaccine data observed previously [23].

3.2. IL-4R antagonist adjuvanted vaccines induce enhanced HIV-specific systemic and mucosal effector and memory CD8+ T cell responses

Next we evaluated the number of $KdGag_{197-205}$ tetramer reactive cells induced by the IL-4C118 antagonist vaccination. Data indicated that i.n. FPV-HIV-IL-4C118/i.m. VV-HIV-IL-4C118 prime-boost immunisation induced significantly greater numbers of $KdGag_{197-205}$ tetramer reactive systemic CD8+ T cells (∼average 20%) (Fig. 2), compared to the control FPV-HIV/VV-HIV prime-boost immunisation (∼average 7%) ($p = 0.0001$). Interestingly, when the adjuvant was delivered only in the prime (Table 1 strategy 2) the magnitude of systemic $KdGag_{197-205}$-specific tetramer reactive cells were very similar to the control vaccination (Fig. 2). However, when the IL-4C118 adjuvant was only delivered in the booster vaccination (Table 1 strategy 3) even though significantly elevated numbers of $KdGag_{197-205}$ tetramer-specific T cells were detected compared to the control or the prime only groups (Fig. 2) ($p = 0.0001$, and $p = 0.018$, respectively), the $KdGag_{197-205}$-specific T cell avidity of i.n. FPV-HIV/i.m. VV-HIV-IL-4C118 prime-boost immunised group was comparable to that of the control vaccine strategy (Fig. 1). These results were similar to what was observed with IL-13Rα2 adjuvanted vaccine strategy [23].

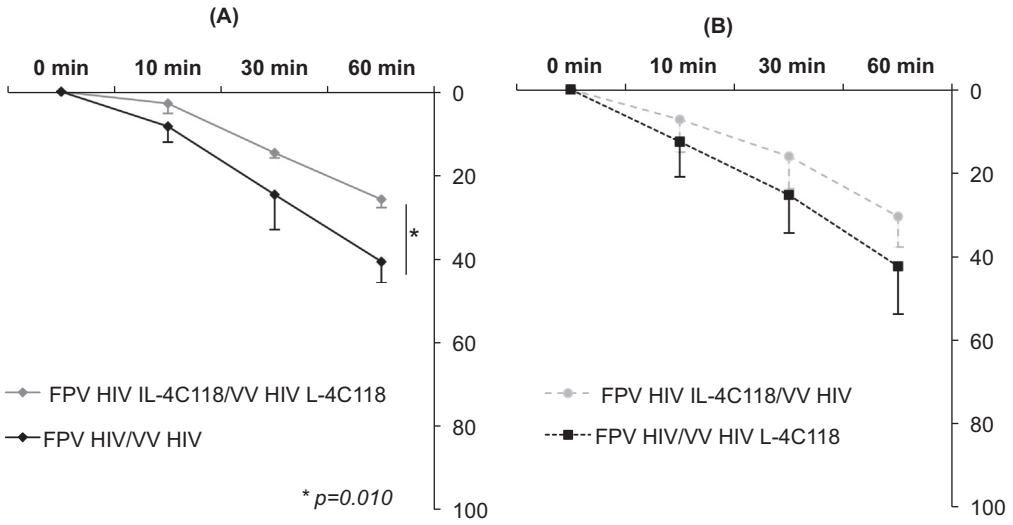

Fig. 1. Avidity of HIV-specific CD8+ T cells following IL-4R antagonist vaccine compared to the control. BALB/c mice ($n = 4$–5 per group) were i.n./i.m. prime-boost immunised with FPV-HIV/VV-HIV and the recombinant pox virus vaccines co-expressing IL-4R antagonist as indicated in Table 1. 14 days post booster immunisation, percentage of $KdGag_{197–205}$ positive CD8+ splenocyte loss (dissociation) was measured as described in material and methods. Graph (A) indicates the IL-4R antagonist used in prime and the booster vaccination (grey line), compared to the control (black). Graph (B) indicates IL-4R antagonist used in the prime only (grey dotted) compared to booster only (black dotted line). Data represent mean ± SD and the p values were calculated using two-tailed, two sample equal variance Student's t-test. FPV-HIV/VV-HIV vs. FPV-HIV-IL-4C118/i.m. VV-HIV-IL-4C118 ($p = 0.010$) and FPV-HIV/VV-HIV vs. FPV-HIV-IL-4C118/i.m. VV-HIV ($p = 0.025$). No significant differences were found between FPV-HIV/VV-HIV vs. FPV-HIV/i.m. VV-HIV-IL-4C118 group.

Furthermore, the ability of HIV-specific CD8+ T cells to produce IFN-γ following $KdGag_{197–205}$ stimulation were evaluated both in systemic (splenic) and mucosal compartments (iliac or genito-rectal nodes) (Fig. 3A and B). Data indicated that i.n. FPV-HIV-IL-4C118/i.m. VV-HIV-IL-4C118 prime-boost immunisation strategy also induced elevated numbers of splenic effector CD8+IFN-γ+ T cells (∼18%) compared to the control vaccine strategy (∼6%) (Fig. 3A and C) measured by ICS. The splenic IFN-γ ICS response pattern was highly consistent with the tetramer data observed in Fig. 2. Our data clearly indicated that our novel IL-4R antagonist vaccine strategy can also induce elevated mucosal HIV-specific CD8+IFN-γ+ T-cell numbers compared to control vaccination (Fig. 3B). Polyfunctional CD8+ T cells are known to correlate with protective immunity, therefore we next assessed the ability of CD8+ T cells to express IFN-γ, TNF-α and IL-2. Interestingly, the data indicated that number of polyfunctional HIV-specific T cells; IFN-γ and TNF-α ($p = 0.021$) (Fig. 3D) and IFN-γ, TNF-α and IL-2 ($p = 0.005$) (Fig. 3E) were also significantly elevated in the IL-4C118 adjuvanted vaccine group compared to the mice that received the control vaccine. Furthermore, the above strategy was also able to induce elevated numbers of $CD8^+IFN\text{-}\gamma^+$ (consistent to our ICS data) and IL-2 effector HIV-specific CD8+ T cells in iliac nodes compared the control vaccine (Fig. 4) as measured by ELISPOT. The evaluation of polyfunctional HIV-specific CD8+ T cells (specifically IL-2) in mucosal sites

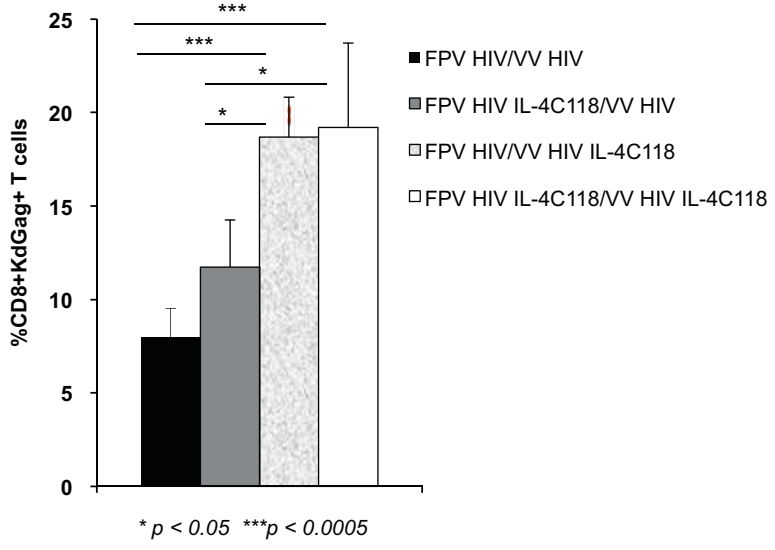

Fig. 2. HIV-specific tetramer reactive CD8+ T cell responses post IL-4C118 adjuvanted vaccination. BALB/c ($n = 5$ per group) were i.n./i.m. prime-boost immunised with FPV-HIV/VV-HIV and/or rFPV or rVV vaccines co-expressing IL-4R antagonist (as indicated in Table 1). 14 days post booster immunisation, percentage of $KdGag_{197–205}$ positive T cells was evaluated in spleen. The data represent mean +SD. All the p values were calculated firstly, using one-way ANOVA to compare the groups and next post hoc analysis was performed with Sidak multiple comparison test using IBM, SPSS (formerly known as Statistical Package for the Social Sciences) statistical software version 21. FPV-HIV/VV-HIV vs. FPV-HIV/VV-HIV IL-4C118 ($p = 0.0001$); FPV-HIV/VV-HIV vs. FPV-HIV IL-4C118/VV-HIV IL-4C118 ($p = 0.0001$); FPV-HIV-IL-4C118/VV-HIV vs. FPV-HIV/VV-HIV IL-4C118 ($p = 0.018$) and FPV-HIV IL-4C118/VV-HIV vs. FPV-HIV IL-4C118/VV-HIV IL-4C118 ($p = 0.016$). These experiments were repeated over three times.

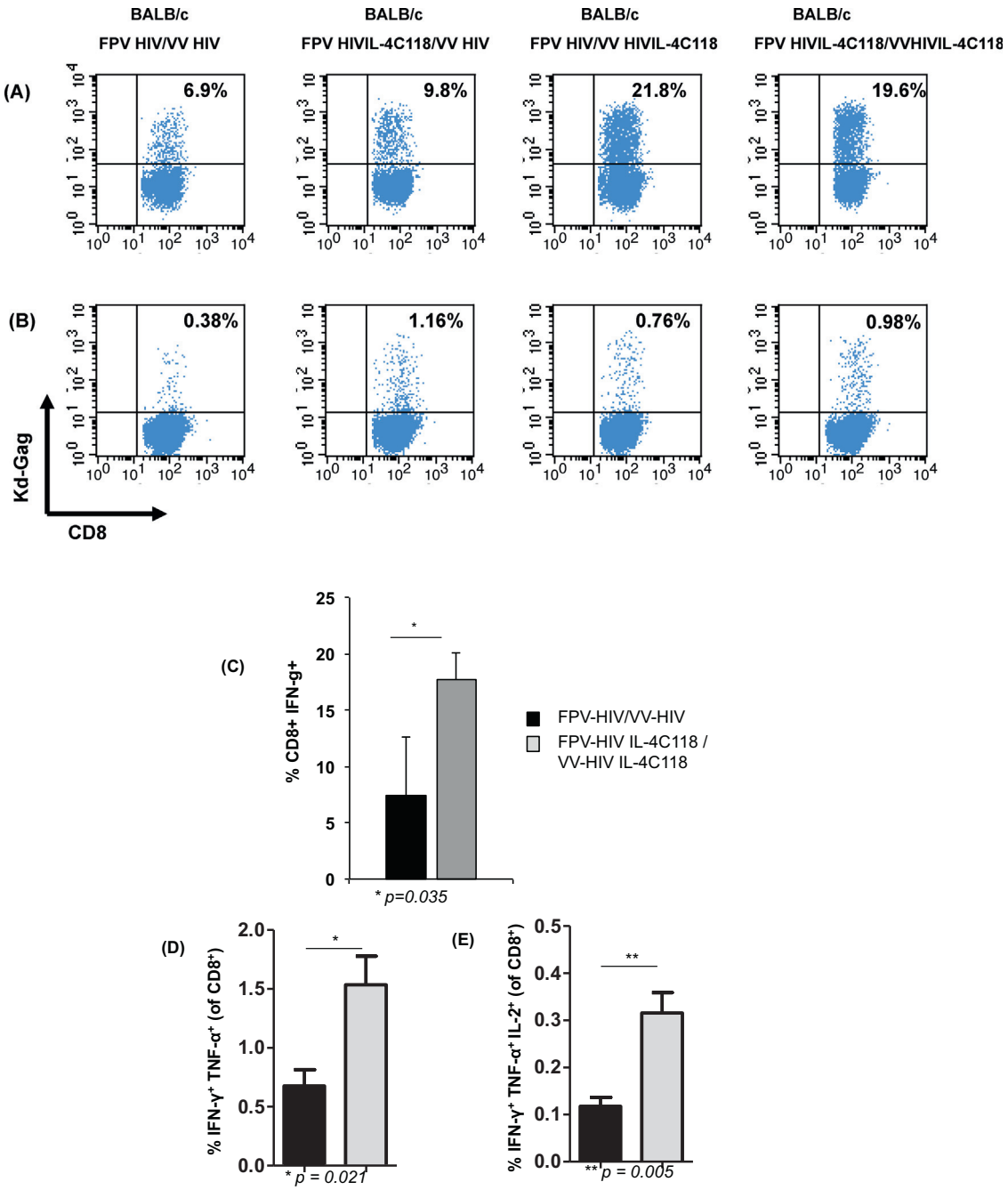

Fig. 3. Cytokine expression by systemic HIV-specific effector CD8+ T cells. Mice ($n = 5$) were immunised i.n./i.m. as in Table 1 (strategies 1, 2, 3 and 4) and at 14 days prime-boost immunisation, IFN-γ, TNF-α and IL-2 protein expression in spleen (A) and genito-rectal nodes or iliac nodes (B) were evaluated by ICS following AMQMLKETI gag peptide stimulation. The graphs represent splenic CD8 T cells expressing IFN-γ only (C) IFN-γ and TNF-α (D) and IFN-γ, TNF-α and IL-2. The data represent mean +SD and the p values were calculated using two-tailed, two sample equal variance Student's *t*-test. These experiments were repeated three times.

(iliac nodes) by ICS is a challenging task due to small sample size. However, we have found that when mucosal HIV-specific CD8+ T cell immunity is evaluated specifically at the gut mucosae at a single cell level using Fluidigm Biomark analysis, the IL-4R antagonist vaccination can induce enhanced expression of many other immunomodulatory cytokines/chemokines, granzymes and perforins compared to the control vaccination [80]. Interestingly, these elevated systemic/mucosal $CD8^+IFN\text{-}\gamma^+$ T cells responses were also found to be long lived as elevated responses were detected at 8 weeks post booster vaccination. Spleen control vaccine vs. IL-4C118 $p = 0.012$ (Fig. 5A and B).

3.3. IL-4R antagonist adjuvanted vaccines induce both excellent serum IgG1 and IgG2 antibody responses

As it is thought that inhibition of Th2 cytokine activity could potentially dampen antibody responses, we also evaluated whether the IL-4C118 antagonist and IL-13Rα2 adjuvanted vaccines can also induce B cell mediated immunity towards HIV Gag. Female BALB/c mice $n = 8$ were immunised i.n./i.m. with the vaccines indicated in Table 1 (strategies 1, 4 and 5), HIV p55 gag specific serum IgG1 and IgG2a antibody responses were evaluated at 3-week intervals for 12 weeks following the booster vaccination (Fig. 6A–C).

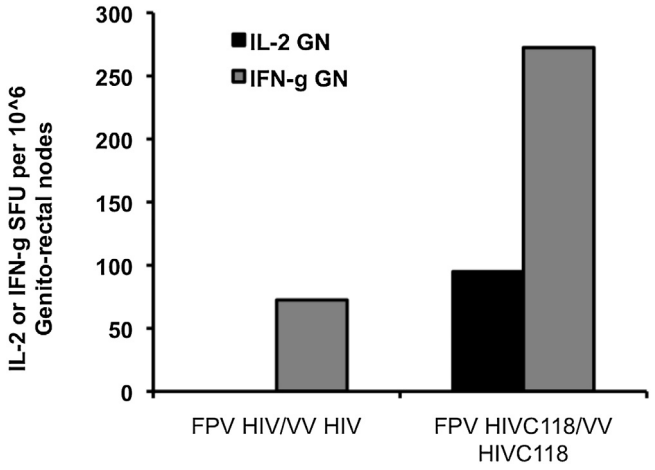

Fig. 4. IFN-γ and IL-2 expression by mucosal HIV-specific CD8+ T cells were evaluated in genito-rectal nodes 14 days post prime-boost immunisation by ELISpot following AMQMLKETI gag peptide stimulation. CD8+IFN-γ+ and CD8+IL-2+ were evaluated and data were plotted after subtracting the background cell counts (which were less than 4–18 SFU). The error bars represent mean ± SD. These experiments were repeated three times.

The absorbance data indicates that the p55-specific IgG1 antibody responses trend generated by all three vaccines were similar across the 12-week period (Fig. 6A). The endpoint titres at 12 weeks were approaching significance ($p = 0.0587$) between the IL-4C118 antagonist and IL-13Rα2 immunised groups (Fig. 6B). Interestingly, the p55-specific IgG2a antibody responses consistently increased following IL-4C118 antagonist vaccine compared to IL-13Rα2 vaccines across the 12-weeks (Fig. 6A and C). The endpoint titres clearly indicated that the IL-4C118 antagonist vaccine could induce significantly higher p55-specific IgG2a antibody titres at 6, 9 and 12 weeks (Fig. 6C). At 6 weeks the control vaccine was also significantly ($p = 0.0256$) higher than the IL-13Rα2 vaccine (Fig. 6C). From the both the absorbance trends and the endpoint titre data it was evident that the IL-13Rα2 vaccine regime has suppressed the induction of p55 IgG2a antibodies while having no significant effect upon IgG1 response, the IL-4C118 antagonist elicited comparable antibody responses to the control vaccine.

3.4. IL-4C118 antagonist induces excellent protective immunity

Finally we assessed the protective efficacy of the novel IL-4C118 vaccine compared to our previously tested IL-13Rα2 adjuvanted and the control vaccines [23], using a surrogate attenuated recombinant influenza virus PR8-$K^dGag_{197–205}$ challenge to evaluate CD8+ T cell mediated immunity. In this study, eight weeks following booster immunisation, mice were challenged intranasally with 75–100 PFU of influenza virus expressing the $KdGag_{197–205}$ immunodominant epitope and body weights were monitored daily for 9–10 days. Mice that received the i.n. FPV-HIV-IL-4C118/i.m. VV-HIV-IL-4C118 vaccination showed better protective efficacy compared the previously tested IL-13Rα2 adjuvanted vaccines [23] (Fig. 7A and B). The IL-4C118 and adjuvanted group showed significantly higher ($p < 0.05$) recovery rates compared to the wild type BALB/c mice that received the control vaccination, specifically at peak influenza infection (Fig. 7A). The above protective data were also consistent with the slower dissociation rates (Fig. 1) the enhanced $K^dGag_{197–205}$ tetramer CD8+ T cell staining (Fig. 2) and the polyfunctional IFN-γ/IL-2 CD8 T cell responses observed in the systemic and mucosal compartments (Fig. 4), following immunisation with the IL-4C118 antagonist vaccine. As shown in Fig. 6, both IgG1 and IgG2a anti-Gag p55 responses were similar between mice immunised with either the control or the IL-4C118 adjuvanted vaccines. Suggesting that antibody had little influence upon the outcome of the PR8-$K^dGag_{197–205}$ challenge and the difference in immune protection observed was determined predominantly by the HIV-Gag specific CD8+ T cell response.

4. Discussion

We have previously demonstrated that the i.m./i.m. poxvirus vectored heterologous prime-boost vaccine strategy induces elevated numbers of HIV-specific CD8+ T cells of lower avidity expressing IL-4 and IL-13 compared to a purely mucosal vaccination [20,21]. These studies also demonstrated that the magnitude of HIV-specific CTLs did not correlate with the avidity measured by MHC-1/CD8 T cell interaction. Using gene knockout mice it was later established that a higher avidity HIV specific CD8+ T cell response can be generated in the absence of IL-13, with enhanced protective efficacy following a surrogate influenza-HIV challenge [23,44] These observations suggested that IL-4 and IL-13 cytokines influenced the induction and/or expansion of the CD8+ T cell

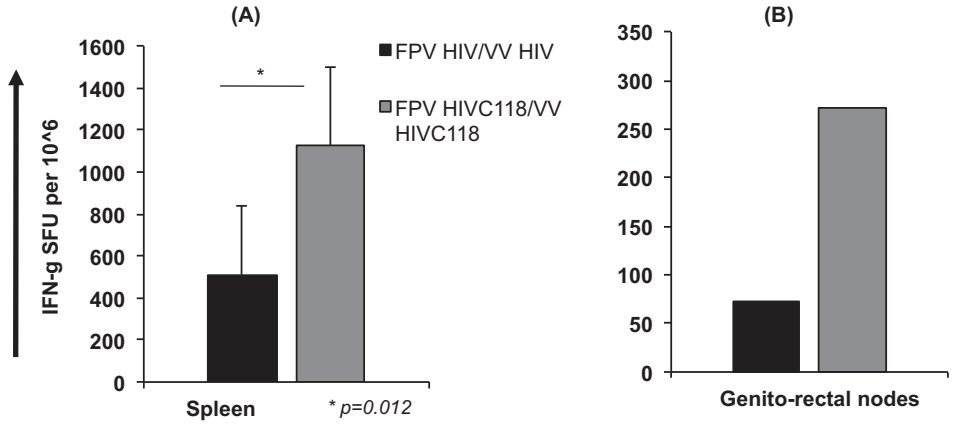

Fig. 5. HIV-specific memory T cell responses post IL-4C118 adjuvanted vaccination BALB/c ($n = 5$ per group) were i.n./i.m. prime-boost immunised with control vaccination FPV-HIV/VV-HIV (black) and FPV-HIV-IL-4C118/VV-HIV-IL-4C118 (grey) as indicated in Table 1. Eight weeks post booster immunisation, single cell suspensions were prepared and $2x10^5$ lymphocytes were cultured in the presence of AMQMLKETI gag peptide in an ELISpots were performed as described in Methods. The graphs represent the IFN-γ responses in spleen (A) and genito-rectal nodes (B). The background cell counts (which 4–10 SFU for IFN-γ ELISpot) were subtracted before plotting the data. Error bars represent mean ± SD and the p values were calculated using two-tailed, two sample equal variance Student's *t*-test. These experiments were repeated three times.

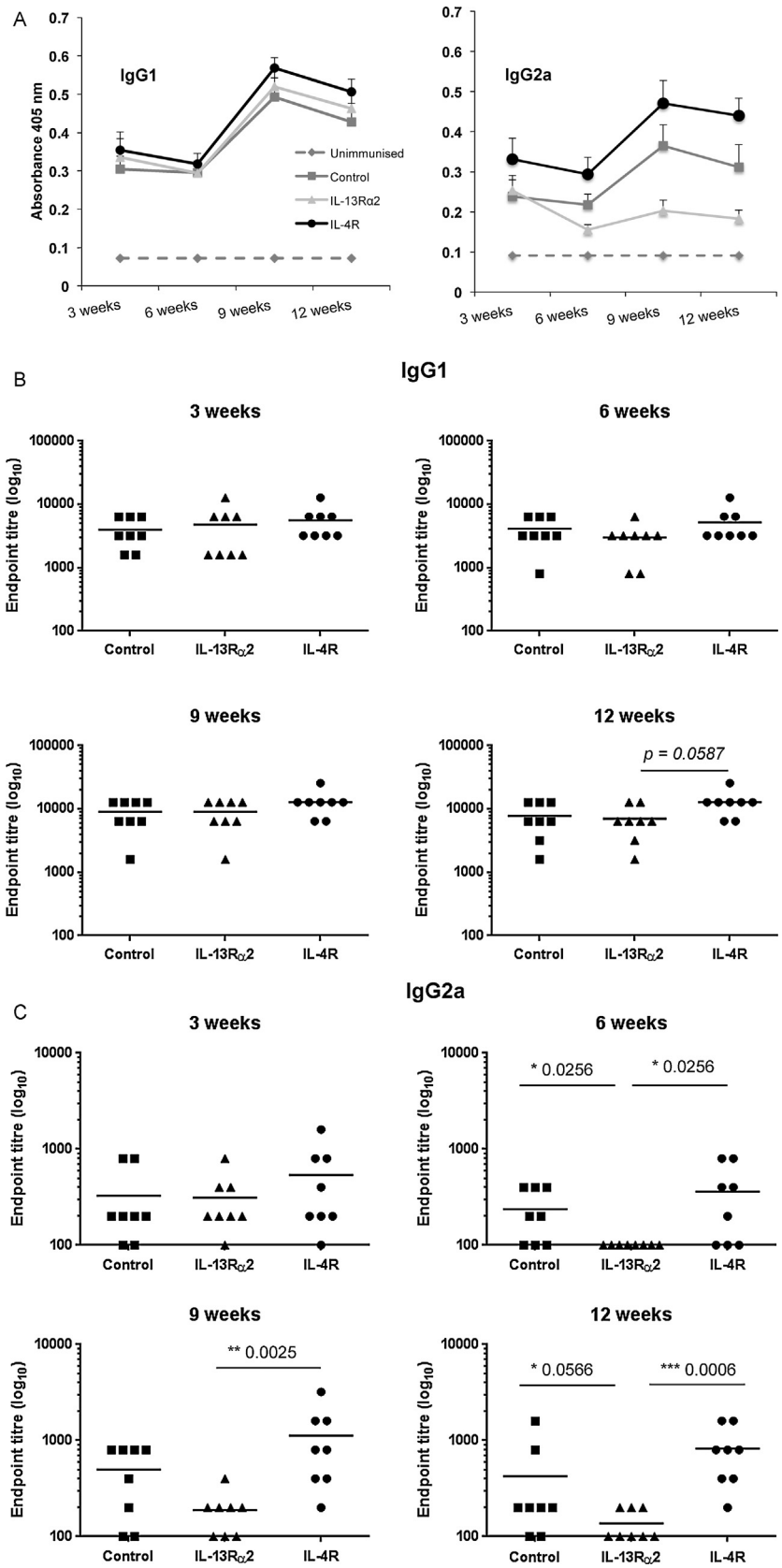

Fig. 6. The p55-gag specific IgG1 and IgG2a antibody trends and endpoint titres following prime-boost immunisation. Female BALB/c mice ($n = 8$ per vaccine group) were immunised with control vaccine FPV-HIV/VV-HIV, FPV-HIV-IL-13Rα2/VV-HIV-IL-13Rα2 or FPV-HIV IL-4C118/VV-HIV IL-4C118 vaccines (regimes 1, 4 and 5, Table 1) and the serum was collected at 3-week intervals following the booster vaccination and analysed for p55-gag specific IgG1 and IgG2a antibodies. (A) Graphs represent the trends of the serum dilutions 1:3200 for IgG1 (left) and 1:400 for IgG2a (right) 3, 6, 9 and 12 weeks post booster vaccination. The *y*-axis indicates the absorbance (wavelength of 405 nm) and the *x*-axis indicates the time intervals post boost immunisation. (B and C) Graphs represent the end point titres. To calculate the p55-gag specific IgG1 (B) and IgG2a (C) endpoint titres, serial serum dilutions were performed and the endpoints were calculated as described in methods statistical analysis section. The errors bars represent the ±SEM and the *p* values were calculated using the Mann–Whitney *U* test. These experiments were repeated two times.

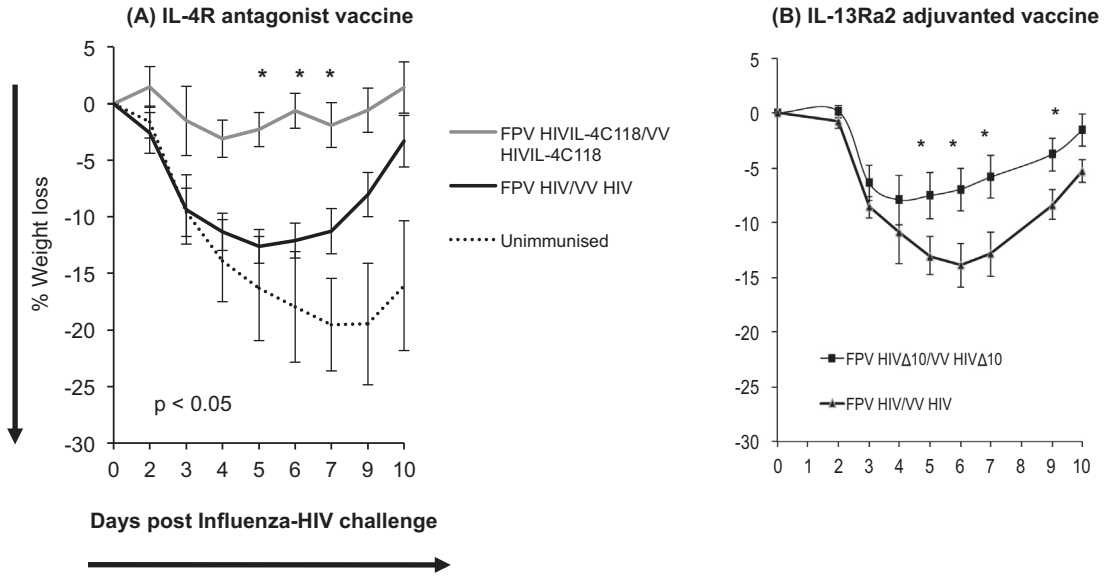

Fig. 7. Protective immunity following PR8-KdGag$_{197–205}$ challenge. BALB/c mice (n = 5–8 per group) were i.n./i.m. prime-boost immunised FPV-HIV/VV-HIV and/or rFPV or rVV vaccines co-expressing IL-4C118 (A left) or IL-13Rα2 (B right) [23]. At 6 weeks post booster immunisation or unimmunised control mice were challenged mucosally (i.n.) with 75–100 units influenza virus PR8 expressing KdGag$_{197–205}$ epitope. Body weights were monitored for 10 days as described in Section 2. The data represent mean ± SEM and *p values at peak of infection calculated using two-tailed, two sample unequal variance Student's t-test. These experiments have been repeated two times.

population following vaccination. The current studies demonstrated that the IL-4C118 adjuvant, an antagonist for both type I/II IL-4R receptors which blocks both IL-4 and IL-13 cell signalling (see Suppl. Diagram 1), included in both the prime and booster HIV vaccine strategy (i) significantly enhanced HIV specific KdGag$_{197–205}$ positive CD8+ T cell response (average 20% of total CD8+ T cells), compared to the non-adjuvant vaccine eliciting average 7% of CD8+ T cells, (ii) induced enhanced numbers of effector and memory mucosal and systemic HIV specific CD8+ T cells that expressed IFN-γ, TNF-α and IL-2 which associated with high avidity T cells of better protective efficacy following a surrogate influenza-KdGag$_{197–205}$ challenge, compared to the control vaccination. Induction of multifunctional T cells expressing IFN-γ, IL-2 and TNF-α is considered a hallmark of high avidity T cells with protective efficacy towards HIV [45–47]. These features are also characteristic of elite controllers of HIV whose HIV specific CD8+ T cells are of high avidity, with elevated multifunctional capacity and viral control [48,49].

Our previous findings indicate that the avidity of the resultant HIV specific CD8+ T cell repertoire was determined during the priming immunisation [23], this is highly consistent with our current findings where delivering the IL-4C118 adjuvant in the boost only, resulted in a major increase in magnitude of the HIV specific T cell response, without significant avidity enhancement. The results presented here and our recent findings indicate that IL-4/IL-13 not only have significant effects during the induction of the immune response but also affect the functions of activated CD8+ T cells which regulated responsiveness to IL-4/IL-13 by reducing cell surface expression of IL-4Rα [50] and also regulating CD8 co-receptor expression with direct effects on the avidity of CD8+ T cells [51]. The inhibition of IL-13 activity by IL-13Rα2 adjuvanted vaccine [23] was shown to significantly up-regulate CD8 co-receptor expression on KdGag$_{197–205}$-specific CD8+ T cells and this correlated with enhanced TCR avidity and poly-functionality [51]. Interestingly, we have also demonstrated that mucosal vaccination induces high avidity HIV-specific T cells with lower IL-4/IL-13 expression and higher CD8-coreceptor densities were detected on K^{d}Gag$_{197–205}$-specific T cells compared to i.m./i.m. delivery [51] On the contrary, co-expression of active IL-4 by a recombinant VV resulted in enhanced IL-4Rα expression, reduced CD8 levels on CD8 T cells, reduced avidity and significantly reduced IFN-γ and TNF-α expression [50,51]. Indeed earlier studies using pathogenic Orthopoxviruses expressing IL-4 were shown to severely curtail the development of effective cytotoxic cell mediated immunity with the mice unable to control infection [52,53]. As the avidity of a CD8+ T cell can change during the course of an infection [54] and similarly the avidities of different CD8 epitopes are know to be vastly different [43], the true efficacy of the these novel vaccine expressing respective receptors should next be evaluated in a non-human primate model following an SIV challenge.

The heterologous FPV-HIV/VV-HIV vaccine strategy was originally designed to elicit a CD8+ T cell mediated immunity towards HIV gag/pol antigen via intracellular processing and MHC-I presentation, however poxviruses can also be good inducers of sustained antibody responses towards viral antigens, one of the features attributed to the long lasting effects of the smallpox vaccine [55]. Previous studies involving co-expression of type-1 cytokines, e.g. IL-2, IL-12, IFN-γ, by viral vaccines to enhance cell-mediated immunity has been associated with reduced serum antibody levels [52,56,57]. Co-expression of type-1 cytokines can also result in variable outcomes, and as shown in several studies can be deleterious to the resultant cell mediated response [20,52,57]. Specifically, inappropriately timed type-1 cytokine expression and polarisation of Th1 immunity in some circumstances can be counterproductive to both cell mediated and humoral responses. Examination of the anti-HIV p55-gag response following control i.n. FPV-HIV/i.m. VV-HIV prime-boost immunisation demonstrated significant levels of both IgG1 and IgG2a in the sera of mice. More surprisingly, following immunisation of mice with the IL-4C118 adjuvant HIV vaccine, which induced enhanced high avidity HIV specific CD8+ T cells with IL-2 and IFN-γ expression also induced elevated HIV p55-gag IgG2a antibody responses six weeks post booster vaccination and was sustained over time.

The recent RV144 trial included both a canarypox virus (very similar to rFPV) expressing gag/pol/env antigens followed by a protein booster to enhance the anti-env humoral response. In that study the 31% protective efficacy observed was linked to antibody-mediated immunity, no cytotoxic CD8 T cell responses

were observed, which may explain the partial protective efficacy. Interestingly, isotype switching and high levels of IgG2 antibodies directed towards the gag protein have been linked to protection, specifically in HIV controllers not carrying the 'protective' human leucocyte antigen HLA B alleles [58]. Although, the mechanism by which gag-specific antibodies provided delayed progressions remains unknown, in some HIV controllers, antibodies have shown to play a role in ADCC [59,60]. It has been thought that production of IFN-γ and gag-specific antibodies particularly IgG2 may provide stimulation of plasmacytoide DC's, which are typically reduced in HIV infected patients but not in controllers [61,62]. These observations suggest that induction of gag-specific antibodies could play a pivotal role in providing the best protection possible against HIV-1. Our IL-4R antagonist vaccine has shown to induce excellent long lasting IgG2a antibody immunity. The induction of both high quality T and robust B cell immunity make our IL-4R antagonist HIV vaccine a good candidate for the future.

Considering the similarity of the T cell responses between the IL-4C118 adjuvant HIV vaccine and our previous IL-13Rα2 adjuvanted vaccine study [23] the majority of the observed effects on the induced quality of HIV specific CD8+ T cell responses are likely due to the inhibition of IL-13 cell-signalling via the type-II IL-4R (IL-4Rα/IL-13Rα1). Sequestration of IL-13 using a decoy IL-13R will reduce IL-13 binding to both type II IL-4R and plasma membrane IL-13Rα2, however IL-4 will still available to engage with type-I/II IL-4R for signalling. In contrast, expression of the IL-4C118 antagonist will block both type-I/II IL-4R to IL-4 and IL-13 mediated signalling, however plasma membrane IL-13Rα2 could still bind free IL-13 (see Suppl. Diagram 1). Although the two different adjuvanted vaccines induced very similar high quality CD8+ T cell responses, the differential cytokine-receptor interactions between the vaccines resulted in significantly different HIV p55-gag IgG2a antibody profile, IL-4C118 antagonist showing elevated responses compared to IL-13Rα2 adjuvanted vaccine. This is suggestive of two possible mechanisms of signalling (i) IL-4 signalling via IL-4Rα is antagonistic to IFN-γ dependent [63] or independent [64] B cell IgG2a isotype class switching retarding both control vaccine and IL-13R adjuvant vaccine IgG2a responses. Whereas, with the IL-4C118 adjuvant vaccine IL-4 is unable to stimulate cell signalling resulting in enhanced and early HIV gag/pol specific IgG2a isotype switching following prime-boost vaccination. (ii) Alternatively, signalling via the IL-13Rα2 receptor in the absence of IL-4Rα signalling can influence B cell maturation and IgG2a class switching during the Th1 influenced humoral response. Collectively, the data indicate that these IL-4/IL-13 receptors are important players in modulating protective immunity.

Our previous studies have shown that rFPV is an excellent mucosal delivery vector compared to rVV [19,20,40] and the priming immunisation determines the avidity of the CD8+ T cell repertoire induced [23]. We have recently completed an analysis of lung-derived DC (LDC) subsets induced 24 h following intranasal immunisation of mice [80]. Interestingly, unlike other pox viral vectors tested rFPV priming was shown to induce a unique $CD11b^{+}$ $CD103^{-}$ LDC population and adoptive transfer studies demonstrated that the unlike $CD103^{+}$ LDC the $CD103^{-}$ LDC population favoured the induction of high avidity CD8 T cells following immunisation. Interestingly, both the IL-13Rα2 and IL-4C118 adjuvant vaccines induced higher numbers of the $CD11b^{+}$ $CD103^{-}$ LDC population relative to the control which correlated with proliferation of high magnitude, strong avidity HIV specific $CD8^{+}$ T cell responses and protective immunity. Differences in $CD11b^{-}$ $B200^{+}$ and $CD11b^{-}$ $CD8^{+}$ LDC subsets were also detected between the IL-13Rα2 and IL-4C118 adjuvant vaccines. These changes in the LDC populations are indicative of the effects of endogenous IL-4/IL-13 are influencing the innate immune response, imprinting the quality of the downstream adaptive cell mediated and humoral immune outcomes [80].

These observations and the current results raise the question; what is the source of IL-13 during the innate response? While IL-13 and IL-4 are traditionally thought to be expressed by Th2 CD4+ cells, recent studies have identified an additional important cellular source of IL-13 early in the immune response. Innate lymphoid cells (ILC) are emerging as central effectors of innate and adaptive immunity and tissue remodelling [65,66]. Activated type 2 ILC (ILC2) cells are critically required for orchestrating Th2 immune responses, expulsion of extracellular parasites, maintaining tissue homeostasis and are the main mediators of allergy and asthma [67–69]. Following injury/infection, epithelial cells release cytokines IL-25 and IL-33 which activate ILC2 cells to express IL-5, IL-9, IL-13, and potentially small amounts of IL-4 [69]. Following intranasal infection of mice with a recombinant influenza A virus, activated ILC2 accumulate in the lung and express not only IL-5, IL-9, IL-13 but also amphiregulin (Areg), the ligand for EGFR which drives epithelial cell proliferation and tissue repair [70]. In the context of an attenuated vaccine similar ILC2 activation and IL-13 expression will have a negative impact upon the resulting quality and magnitude of the Th1 anti-viral response. Potential additional sources of IL-4 during innate responses may include stimulation of basophils [71] and activated iNKT2 cells [72].

Poxviruses devote a large proportion of the genomic information to express factors that modulate and evade the host's antiviral innate and adaptive immune responses [73]. Of particular relevance to this study are factors secreted from pox virus infected cells which modulate the balance of Th1 and Th2 immunity. VV is known to express soluble type-I and type-II IFN binding proteins which sequester IFN-α and IFN-γ, respectively [74,75] VV also expresses soluble high affinity decoy receptors for TNF-α, and IL-18 which bind and prevent these cytokines from interacting with the natural receptors [76,77]. Poxviruses apply significant resources into reducing the activity of these antiviral cytokines which are required for activation of type-1 ILC i.e. type-I IFNs and IL-18, or neutralise the major secreted antiviral products, i.e. IFN-γ, TNF-α. IL-18 is critical for strong antiviral Th1 immunity, indeed with $IL\text{-}18^{-/-}$ mice the immune response following poxvirus infection is screwed towards a Th2 cytokine profile (enhanced IL-4 and IL-10), reduced cytotoxic NK and CD8+ T cell responses and enhanced populations of suppressive Treg cells [78]. Recent studies have demonstrated that deletion of the MVA IL-18BP gene can significantly enhance the efficacy of MVA vectored vaccines with increases in the HIV specific CD8+ and CD4+ T cell populations following immunisation [79].

In conclusion, our data indicate that transiently neutralising of IL-13 activity specifically at the priming cell milieu can significantly improve the avidity of the resulting HIV specific CD8+ T cell responses. However, the transient co-neutralisation of both IL-4 and IL-13 activity at the vaccination site is greatly beneficial in the induction of both gag-specific IgG1 and IgG2a antibody immunity, unlike the IL-13Rα2 adjuvanted vaccine that only has the capacity to induce IgG1 antibodies while inhibiting IgG2a. This suggests either IL-4 cell-signalling in the absence of IL-13 delays gag-specific antibody isotype class switching, highlighting the importance of the IL-4R/IL-13R complex in modulating antibody immunity or IL-13Rα2 signalling plays a role in modulating antibody immunity which warrants further investigation. Our data clearly demonstrate that the inclusion of an IL-4/IL-13 antagonist has excellent potential to induce a more balanced immune outcome inducing elevated high quality mucosal and systemic CD8 T cell and also B cell immunity. This offers exciting prospects for a future HIV vaccine development as well as other chronic infections that which require efficacious Th1 mediated immunity for prevention and control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.08.023.

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